Design and Synthesis of Fluorescent Pilicides and Curlicides: Bioactive Tools to Study Bacterial Virulence Mechanisms

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Abstract: Pilicides and curlicides are compounds that block the formation of the virulence factors pili and curli, respectively. To facilitate studies of the interaction between these compounds and the pili and curli assembly systems, fluorescent pilicides and curlicides have been synthesized. This was achieved by using a strategy based on structure–activity knowledge, in which key pilicide and curlicide substituents on the ring-fused dihydrothiazolo 2-pyridone central fragment were replaced by fluorophores. Several of the resulting fluorescent compounds had improved activities as measured in pili- and curli-dependent biofilm assays. We created fluorescent pilicides and curlicides by introducing coumarin and 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) fluorophores at two positions on the peptidomimetic pilicide and curlicide central fragment. Fluorescence images of the uropathogenic Escherichia coli (UPEC) strain UTI89 grown in the presence of these compounds show that the compounds are strongly associated with the bacteria with a heterogeneous distribution.

Keywords: antivirulence • biological activity • coumarin • fluorescence • structure–activity relationships

Introduction

Multidrug resistant (MDR) bacterial strains present a growing global health problem. As a consequence, the search for new antibacterial agents and new methods to deal with bacterial resistance is urgent.[3] Toward this end, understanding the details of the uropathogenic E. coli (UPEC) pathogenic cascade is revealing ways to target critical pathways to develop anti-virulence therapeutics. We have discovered that type-I pili, an adhesive pili assembled by the chaperone/usher pathway (CUP), play an essential role in invasion of bladder cells and in the formation of biofilm-like intracellular bacterial communities (IBCs) that protect bacteria from host defenses and antibiotics.[2–5] Further, CUP pili play a critical role in biofilm formation, mediating not only interactions with host tissue, but also colonization of catheters and other surfaces in nosocomial settings. Also, an amyloid fiber called curli is critical in UPEC biofilm formation and the molecular machine that mediates curli assembly has been dissected.[6–11] Our understanding of the structural basis of CUP pili biogenesis has led to the design of pilicides that bind to the chaperone and block critical functions thus preventing pilus assembly.[6–11] Numerous (~100) CUP systems are now known to be encoded in gram-negative genomes[12] but little is known about their function. A typical E. coli genome encodes approximately 10 such systems.[13] Further detailed studies of the function and regulation of CUP pili and other extracellular fibers are an important route to understanding the bacterial adaptation and survival strategies that may be particularly relevant to human infections and providing targets for the development of new therapeutics. Here we describe the development of new compounds that will serve as a strong foundation to support investigation of novel anti-fiber therapeutics targeting critical assembly and adhesion functions of fibers required for the determination of tropism and the organization of bacterial communities during infection.

Ring-fused 2-pyridones are peptidomimetics that can target protein–protein interactions in macromolecular assembly. We have previously shown that ring-fused dihydrothiazolo 2-pyridones (1) provide an excellent central fragment for design and synthesis of compounds that block the formation of pili and curli.[10,11,14] Pilicides (2a,b) are compounds that block pilus biogenesis (exemplified in UPEC), whereas curlicides (such as 3) prevent curli fiber biogenesis. Development of traceable pilicides and curlicides could potentially be obtained using a biomolecular labeling strategy, for example, by radiolabeling or by the introduction of a fluorescent label. In the latter case, a fluorescent probe is usually attached by using a linker to the biomolecule to avoid any interference with the biomolecular interactions. The low molecular weight of ligands such as the pilicides 2a,b and curlicide 3, implies that this technique could change the overall molecular composition to a great extent and thereby potentially reduce the bioactivity of these compounds. An alternative approach would be to replace key substituents for bioactivity by a fluorophore. To increase the likelihood of succeeding by using this approach, and thus both retain the biological effect and gain fluorescent properties, the structure–activity knowledge on the central fragment could...
be used for both the choice of fluorophore and its positioning on the central fragment. One potential problem with this method is a higher probability for fluorescent quenching of the fluorophore due to its close proximity to the bioactive central fragment. Even so, exchange of one of the substituents on the peptidomimetic central fragment 1 to a fluorophore could render attractive compounds for uptake/distribution studies, development of competition-based assays, Förster resonance energy transfer (FRET) studies on binding interactions, and to specifically image conserved pili and curli assembly machineries in bacterial populations. Initial studies of the structure–activity relationships on the ring-fused dihydrothiazolo 2-pyridone central fragment have shown that the C7 and C8 positions are highly important for bioactivity and should preferably carry larger lipophilic substituents.[10,11,15–17] Consequently, we have in the present study exchanged the substituents in the C7 and C8 positions (Figure 1). The use of these particular fluorophores could be justified by their good (compound 4) to high (compound 5) quantum yields, absorption/emission wavelengths, lipophilicity, lack of net ionic charge, photostability, different emission colors and relatively small size.[18–20]

The synthesis of the ring-fused dihydrothiazolo 2-pyridone central fragment 1 (R = H or Li for biological activity), pilicide (2a), curlicide (3), coumarin fluorophore 4, BODIPY fluorophore 5. Synthesis of the thiazolo ring-fused 2-pyridone central fragment (1a) is performed by using 2-thiazolines (6) and Meldrum’s acid derivatives (7).

This approach, coumarins could also be introduced directly on a bromomethyl-substituted central fragment using deprotected 4-methyl coumarins. In total, 14 new fluorophore-substituted derivatives of the central fragment 1 have been synthesized and the photophysical measurements of these compounds revealed compounds with high quantum yields. In addition, biological evaluation of these compounds as pilicides and curlicides showed a great biological effect of several compounds, with some being both potent inhibitors of pili- and curli-dependent biofilm formation and having fluorescent properties. Finally, treatment of the UPEC strain UTI89 with the compounds under pili producing conditions shows that the compounds are associated to the bacteria and seem to discriminate between different bacteria in a population.

**Results and Discussion**

Coumarins substituted with electron-donating groups in position 7 such as 7-methoxy and 7-diethylamino coumarins are frequently used fluorophores (Figure 1). The linker to the pilicide/curlicide central fragment was preferred through the 4 position on the coumarins to resemble the geometry of the C7 naphthyl substituent in 2 and 3. Consequently, the 7-methoxy coumarin-4-yl acetic acid was first coupled to its corresponding acyl Meldrum’s acid derivative 8 using standard conditions.[10] On the basis of previous structure–activity relationships of the pilicide/curlicide central fragment, a phenyl,[9,15] a 3-trifluoromethylphenyl,[10,22] a 2-thiophenyl,[17] and a cyclopropyl[9,15] were selected as substituents on the Δ2-thiazolines (9a–d). Reacting 8 with the 9a–d in the acyl ketene imine cyclocondensation gave coumarin-substituted thiazolo ring-fused 2-pyridones 10a–d in 68–86% yield (Scheme 1). Compounds 10a–d were next subjected to hydrolysis to give the corresponding carboxylic acids 11a–d in 53–66% yield.

![Scheme 1](image-url)
Table 1. The fluorophore-substituted compounds photophysical properties and abilities to inhibit pili- and curli-dependent biofilm formation.

<table>
<thead>
<tr>
<th>ID</th>
<th>R¹ (C8)</th>
<th>R² (C7)</th>
<th>X</th>
<th>EC₅₀ₐ [µM] Pili</th>
<th>EC₅₀ₐ [µM] Curli</th>
<th>λₘₐₓ [nm]</th>
<th>λₜₕₐₜ [nm]</th>
<th>Quantum Yield [%] (λₜₕ nm)</th>
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<tr>
<td>11a</td>
<td></td>
<td></td>
<td>-S-</td>
<td>&gt; 200</td>
<td>NA</td>
<td>328</td>
<td>394</td>
<td>5 (330)</td>
</tr>
<tr>
<td>11b</td>
<td></td>
<td></td>
<td>-S-</td>
<td>&gt; 200</td>
<td>NA</td>
<td>330</td>
<td>420</td>
<td>1 (330)</td>
</tr>
<tr>
<td>11c</td>
<td></td>
<td></td>
<td>-S-</td>
<td>&gt; 200</td>
<td>NA</td>
<td>328</td>
<td>396</td>
<td>0.7 (330)</td>
</tr>
<tr>
<td>11d</td>
<td></td>
<td></td>
<td>-S-</td>
<td>&gt; 200</td>
<td>NA</td>
<td>328</td>
<td>413</td>
<td>0.4 (330)</td>
</tr>
<tr>
<td>16a</td>
<td></td>
<td></td>
<td>-S-</td>
<td>65</td>
<td>175</td>
<td>329</td>
<td>430</td>
<td>0.6 (355)</td>
</tr>
<tr>
<td>16b</td>
<td></td>
<td></td>
<td>-S-</td>
<td>156</td>
<td>NA</td>
<td>327</td>
<td>393</td>
<td>0.5 (330)</td>
</tr>
<tr>
<td>16c</td>
<td></td>
<td></td>
<td>-S-</td>
<td>18</td>
<td>25</td>
<td>393</td>
<td>474</td>
<td>15 (390)</td>
</tr>
<tr>
<td>16d</td>
<td></td>
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<td>5</td>
<td>17</td>
<td>396</td>
<td>478</td>
<td>11 (346)</td>
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<tr>
<td>28</td>
<td></td>
<td></td>
<td>-S-</td>
<td>4</td>
<td>14</td>
<td>506</td>
<td>524</td>
<td>10 (470)</td>
</tr>
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</table>

These results may be due to a quenching effect of the fluorophore by the neighboring dihydrothiazolo ring-fused 2-pyridone pillar/curlicide central fragment. In an attempt to circumvent this, the linker between the fluorophore and the 2-pyridone central fragment was increased by a one carbon extension. This was realized by using deprotonated 4-methyl coumarins as nucleophiles on bromomethyl-substituted 2-pyridone central fragments. By using this strategy the introduction of both a 7-methoxy-substituted coumarin and a 7-diethylamine-substituted coumarin could be accomplished (Scheme 2). The intermediate bromomethyl-substituted 2-pyridone central fragments (13a and 13b) were synthesized following a previously published procedure.[23] Addition of lithiated coumarins (14a and 14b) to 13a and 13b generated the one carbon extended coumarin-substituted ring-fused 2-pyridones 15a–d in 74–83% yield. Subsequent hydrolysis rendered 16a–d in high yields (81–91%; Scheme 2).

The one carbon extended linker did not influence the quantum yield (16a, $\Phi_F = 0.6\%$ and 16b $\Phi_F = 0.5\%$; Table 1). However, replacing the 7-(methoxy)coumarin by a 7-(diethylamino)coumarin as in 16c and 16d increased the quantum yields ($\Phi_F = 15$ and 6%, respectively, Table 1) and gave C7 coumarin-substituted compounds with useful fluo-

### Table 1. (Continued)

<table>
<thead>
<tr>
<th>ID</th>
<th>$R^1$ (C8)</th>
<th>$R^2$ (C7)</th>
<th>X</th>
<th>EC$_{50}$</th>
<th>EC$_{50}$</th>
<th>$\lambda_{ex}$</th>
<th>$\lambda_{em}$</th>
<th>Quantum Yield</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>[a]</td>
<td>[a]</td>
<td>[b] [c]</td>
<td>[b] [d]</td>
<td>[$\Phi_F$ [%]]</td>
</tr>
<tr>
<td>31</td>
<td>-S-</td>
<td>-S-</td>
<td>14</td>
<td>12</td>
<td>502</td>
<td>514</td>
<td>67 (470)</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>-S-</td>
<td>-S-</td>
<td>10</td>
<td>24</td>
<td>498</td>
<td>509</td>
<td>11 (480)</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>-O-</td>
<td>-O-</td>
<td>13</td>
<td>14</td>
<td>497</td>
<td>531</td>
<td>27 (480)</td>
<td></td>
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<tr>
<td>38</td>
<td>-S(O)-</td>
<td>-S(O)-</td>
<td>29</td>
<td>40</td>
<td>498</td>
<td>516</td>
<td>71 (480)</td>
<td></td>
</tr>
</tbody>
</table>

[a] Estimated from 16–32 data points on every concentration. [b] All substances were dissolved in DMSO and subsequently diluted in phosphate buffer at pH 7.0. The samples DMSO concentrations never exceed 5 wt%. The sample concentrations in the DMSO stock solutions are adjusted so that the final samples never have a peak absorbance higher than 0.1. [c] Wavelengths of the peak absorption. [d] The peak fluorescence. [e] Not active. [f] Reference: POPOP in MeOH. [g] Reference: Perylene in cyclohexane. [h] Reference: Rhodamine 6G in water.
rescent properties. As a consequence, the 7-(diethylamino)-coumarin was used in the introduction of a coumarin in the C8 position of the pilicide/curlicide central fragment. The coumarin-substituted $\Delta^2$-thiazoline 20 was synthesized in three steps starting from 14b and ethyl 2-bromoacetate (Scheme 3).[24] Subsequent reaction with acyl Meldrum’s acid derivative 21 rendered the C8 coumarin-substituted central fragment 22 in 73% yield (Scheme 3). After hydrolysis of 22 into the target compound 23 the photophysical properties were evaluated. From this a quantum yield comparable with the other 7-(diethylamino)coumarin-substituted compounds was observed for 23 ($\Phi_F = 11\%$, Table 1). Thus, compounds with a coumarin fluorophore in both the C7 and C8 positions of the pilicide/curlicide central fragment have been synthesized.

To increase the probability of identifying a bioactive compound with useful fluorescence properties, the possibility of introducing a different fluorophore on the central fragment was investigated. 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY) is a known fluorophore that normally gives high quantum yields, carries no net charges, is relatively insensitive to the choice of solvent, and should give a different emission color as compared to the coumarins.[20] The synthesis of the BODIPY core structure is often accompanied by low yields. The desired 8-propanoic acid-functionalized 1,3,5,7-tetramethyl-substituted BODIPY (24) has previously been synthesized in 21% yield.[25] From 24, the introduction of a BODIPY substituent in the C8 position of the pilicide/curlicide central fragment could be pursued. Coupling 24 using a standard coupling procedure with methylester-protected cysteine gave the intermediate 25 in 64% yield (Scheme 4). Ring closure of 25 to give 26 followed by acyl-ketene imine cyclocondensation with Meldrum’s acid derivative 21 gave the BODIPY-substituted dihydrothiazolo ring-fused 2-pyridone 27 in 64% yield. The following hydrolysis proved to be problematic and the harsh conditions needed for this transformation ultimately gave 28 in 29% yield as a racemate (Scheme 4). However, on the basis of previous reports showing that both enantiomers of the pilicide central fragment are biologically active, it was still entirely possible that racemic 28 could display interesting bioactivity.[9,26]

The photophysical evaluation of 28 gave a quantum yield of 10%, which is surprisingly low for a BODIPY-substituted compound (Table 1). We hypothesized that photo-quenching was a possible reason for this and thus the use of an aryl linker between the fluorophore and the pilicide/curlicide central fragment could circumvent the problem. This would not only increase the distance between the fluorophore and the pilicide/curlicide central fragment but it would also restrict the rotation of the 1,3,5,7-tetramethyl-substituted BODIPY, which is known to generate higher quantum yields.[20] Exchange of the methylene linker for a phenyl results in the need for a revised synthetic approach. A recent publication shows that it is possible to introduce a benzoic acid in the C8 position of the pilicide/curlicide central fragment by Suzuki–Miyaura couplings.[27] From this benzoic acid derivative, the transformation into the desired C8 BODIPY-substituted central fragment seemed feasible. Consequently, 2-pyridone 29 was treated with oxalyl chloride followed by reaction with 2,4-dimethylpyrrole in the presence of BF$_3$·OEt$_2$ and triethylamine (Scheme 4).
amine to give the desired C8 BODIPY-substituted central fragment 30 in 15% yield. Subsequent hydrolysis was straightforward for this BODIPY derivative, giving the corresponding carboxylic acid 31 in 84% yield (Scheme 5). As hypothesized, introduction of the phenyl spacer in the C8 position increased the quantum yield of 31 to a satisfactory 67% (Table 1).

With these two C8 BODIPY-substituted compounds (28 and 31) in hand, introduction of BODIPY in position C7 on the central fragment was next investigated. Coupling the BODIPY carboxylic acid 24 with Meldrum’s acid using standard coupling conditions and a simple MeOH trituration as purification gave the BODIPY-substituted acyl Meldrum/C29s acid derivative 32 in 82% yield (Scheme 6). From this versatile intermediate the following acylketene imine cyclocondensation to the C7 BODIPY substituted pilicide/curlicide tile intermediate the following acylketene imine cyclocondensation to the C7 BODIPY substituted pilicide/curlicide intermediate was performed in 88% yield. Sequen...
mated EC$_{50}$ of 14 µm and is thus a potent dual pilicide–curlicide. This should be compared with the best reported di-substituted compound 3 (known as FN075), which exhibited EC$_{50}$ values of 17 µm in pili-dependent biofilm inhibition and 38 µm in curli-dependent biofilm inhibition (Table 1). The phenyl spacer in 31 resulted in a slight decreased pilicide activity compared to the methylene linker in 28, but is still in the same range as 3. The C7 BODIPY-substituted derivatives all displayed good inhibitory properties of pili- and curli-dependent biofilm formation. The sulfide (34) and oxygen analogue (37) are more potent than the sulfoxide (39) both as pilicides and curlicides (Table 1). However, the fact that the sulfoxide retains much of the biofilm-inhibition activity might be of importance from a therapeutic and drug metabolism perspective. For the coumarin-substituted compounds with ethylene linker (16a–d), the 7-(diethylamino)-coumarins 16c,d are more active pilicides than the 7-(methoxy)coumarins 16a,b (Table 1). Furthermore, the compounds having an ethylene linker are more potent pilicides than the compounds with a methylene linker (16a and 16b compared with 11b and 11d). None of the four 7-(methoxy)-coumarins with a methylene linker 11a–d showed any activity (Table 1).

Finally, to study the compounds distribution in a bacterial population, UPEC strain UTI89 was grown under pili-producing conditions (24 h at 37°C static) with compounds 28 and 34 and examined by fluorescence microscopy (Figure 2). Compounds 28 and 34 appeared to bind bacteria differently, with 34 appearing more punctate and 28 appearing more diffuse. Further experiments are working to examine the reason for these observed differences. We know that even under pilus-inducing conditions, not all bacteria are expressing pili. Thus it is likely that the pilicide is only binding bacteria expressing pili. Experiments are underway to confirm this hypothesis.

**Conclusion**

We have herein developed compounds that are both fluorescent and highly active inhibitors of pili- and curli-dependent biofilm formation. The synthesis of these compounds was initiated to facilitate profound studies of the pilicides and curlicides and to gain information about the complex systems involved in the formation of pili and curli. To create these fluorescent and bioactive compounds we implemented a strategy based on structure–activity information, in which important substituents for bioactivity on the pilicide/curlicide central fragment were replaced by fluorophores. Synthetic methods were developed to enable a total of 14 compounds with either a coumarin or a BODIPY motif in the C7 or the C8 position. Consequently, new interesting intermediates and new reactions to introduce BODIPY fluorophores (e.g., via Meldrum’s acid derivatives and subsequent acyl ketene cyclocondensations) have been developed. Photo-quenching was frequently observed but its origin was elucidated and circumvented to give compounds with useful fluorescence properties. Biological evaluation using whole bacterial pili- and curli-biofilm assays revealed several compounds that are both fluorescent and highly active. Finally, treatment of UPEC strain UTI89 with the compounds under pili-producing conditions shows that the compounds are associated to the bacteria with a heterogeneous distribution over a population. The use of these compounds to study the biological systems they interact with, for example, the relation between the observed staining pattern of the compounds and the heterogeneous distribution of pili production in bacterial populations, is a matter of future studies within our laboratories.

**Experimental Section**

**General synthesis:** All reactions were carried out under an inert atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. CH$_2$Cl$_2$ and 1,2-dichloroethane (DCE) and was distilled from calcium hydride and THF was distilled from potassium. DMF was distilled and dried over 3 Å molecular sieves. All microwave reactions were carried out in a monomode reactor (Smith Synthesizer, Biotage AB) using Smith process vials sealed with a Teflon septa and an aluminum crimp top. Reaction times refer to the irradiation time at the target temperature not the total irradiation time. The temperature was measured with an IR sensor. Flash column chromatography (eluents given in brackets) employed normal phase silica gel (Matrex, 60 Å, 35–70 µm, Grace Amicon). The $^1$H and $^{13}$C NMR spectra were recorded at 298 K with a Bruker DRX-400 spectrometer in CDCl$_3$ (residual CHCl$_3$ ($\delta_1 = 7.26$ ppm) or CDCl$_3$ ($\delta_1 = 7.70$ ppm) as internal standard), [D$_6$]DMSO (residual [D$_6$]DMSO ($\delta_1 = 2.50$ ppm) or [D$_6$]DMSO ($\delta_1 = 40.0$ ppm) as internal...
(3R)-7-(2-(7-Methoxy-2-oxo-2H-chromen-4-yl)methyl)-5-oxo-8-phenyl-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylic acid (11a): LiOH (0.1 m, 1.5 mL, 1 equiv) was added dropwise to a stirred solution of 10a (71.3 mg, 0.15 mmol, 1 equiv) in THF/MeOH (2 mL, 4:1). The reaction mixture was stirred for two hours at room temperature before being concentrated. Purification by silica gel chromatography (CHCl3/MeOH/AcOH, 90:8:2) and then lyophilized from MeCN/H2O (1:3) to give 11a (47 mg, 66%). 1H NMR (400 MHz, [D6]DMSO): δ = 3.49 (dd, J = 1.79 Hz, J = 11.82 Hz, 1H), 3.72-3.88 (m, 6H), 5.48 (dd, J = 1.63 Hz, J = 9.01 Hz, 1H, 5.93 (s, 1H), 6.01 (s, 1H), 6.88 (dd, J = 2.50 Hz, J = 8.83 Hz, 1H), 6.96 (d, J = 2.49, 1H), 7.20-7.28 (m, 1H), 7.29-7.36 (m, 2H), 7.36-7.44 ppm (m, 3H). 13C NMR (100 MHz, [D6]DMSO): δ = 32.4, 35.7, 56.8, 64.1, 109.2, 112.8, 113.1, 115.0, 115.3 (2C), 127.0, 129.2, 129.8 (2C), 130.8 (broad, 2C), 136.8, 149.6, 150.8, 154.0, 155.8, 160.7, 168.3, 163.3, 170.4 ppm; IR: ν = 3422, 1609, 1486, 1207, 1145, 1022, 987, 840, 705 cm⁻¹; HRMS (EI): m/z: calcd for C35H26N2O6S: 544.1042 [M+H]+; found: 544.1049.

(3R)-7-(2-(7-Methoxy-2-oxo-2H-chromen-4-yl)methyl)-5-oxo-8-(3-trifluoromethyl)phenyl-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylic acid (11b): Prepared according to the procedure described for compound 11a starting from 10b (49 mg, 0.09 mmol, 1 equiv), giving 11b (10 mg, 58%). 19F NMR (400 MHz, CDCl3): δ = −4.0 (c = 0.15 in CHCl3); 1H NMR (400 MHz, [D6]DMSO)/CDCl3: 1: δ = 3.60-3.68 (m, 1H), 3.68-3.78 (m, 3H), 3.83 (s, 3H), 5.57 (d, J = 8.11 Hz, 1H). 5.97 (s, 1H). 6.22 (s, 1H), 6.73-6.81 (m, 2H), 7.21 (d, J = 8.73, 1H), 7.39-7.61 ppm (m, 5H); 13C NMR (100 MHz, [D6]DMSO/CDCl3): δ = 33.1, 35.8, 56.2, 66.4, 101.5, 112.6, 112.8, 113.3, 115.6, 115.9, 124.3 (q, J = 272.2 Hz), 125.9, 126.0, 127.4 (d, J = 9.13 Hz), 130.4, 131.9 (q, J = 32.42 Hz), 134.2 (d, J = 34.73 Hz), 137.1, 150.9, 151.0, 154.1, 155.8, 162.3, 162.7, 163.3, 172.5 ppm; IR: ν = 1716, 1709, 1484, 1433, 1388, 1330, 1288, 1211, 1162, 848, 705 cm⁻¹; HRMS (EI): m/z: calcd for C25H15F4NO5S: 530.0885 [M+H]+; found: 530.0880.

(3R)-7-(2-(7-Methoxy-2-oxo-2H-chromen-4-yl)methyl)-5-oxo-8-(thiophen-2-yl)-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylic acid (11c): Prepared according to the procedure described for compound 11a starting from 10c (72.2 mg, 0.15 mmol, 1 equiv) to give 11c (58 mg, 55%). 1H NMR (400 MHz, [D6]DMSO): δ = 3.51 (dd, J = 1.77 Hz, J = 11.61 Hz, 1H), 3.76-3.83 (m, 3H), 3.85 (s, 3H), 5.43 (d, J = 1.52 Hz, J = 9.16 Hz, 1H). 5.91 (s, 1H). 6.04 (s, 1H). 6.91 (dd, J = 2.56 Hz, J = 8.88 Hz, 1H). 6.98 (d, J = 2.54 Hz, 1H), 7.03-7.07 (m, 2H), 7.48 (d, J = 8.88 Hz, 1H), 7.76-7.60 ppm (m, 1H). 13C NMR (100 MHz, [D6]DMSO): δ = 32.6, 35.8, 56.9, 65.1, 101.9, 107.1, 112.78, 112.81, 113.1, 115.5, 126.8, 126.4, 129.8, 130.4, 130.6, 131.8, 152.5, 154.2, 155.8, 160.7, 160.9, 163.3, 170.4 ppm; IR: ν = 1712, 1646, 1608, 1481, 1348, 1388, 1280, 1207, 1145, 1018, 987, 840, 705 cm⁻¹; HRMS (EI): m/z: calcd for C20H16N3O4S: 485.0576 [M+H]+; found: 485.0587.

(3R)-8-Cyclopropyl-7-(2-(7-Methoxy-2-oxo-2H-chromen-4-yl)methyl)-5-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylic acid (16b): Prepared according to the previously published procedure,(15a) starting from 15d (25 mg, 0.056 mmol) and with CHCl3 was hydrolyzed to its corresponding carboxylic acid 16b (91% yield). 1H NMR (400 MHz, CDCl3): δ = 0.56-0.66 (m, 2H), 0.85-1.02 (m, 2H), 1.50-1.60 (m, 1H), 2.96-3.16 (m, 4H), 3.64-3.74 (m, 1H), 3.75-3.83 (m, 1H), 3.87 (s, 3H), 5.65-5.73 (m, 1H), 6.14 (s, 1H), 6.41 (s, 1H), 6.81-6.91 (m, 2H), 7.51-7.59 (m, 1H), 8.9-9.4 ppm (bs, 1H). 13C NMR (100 MHz, CDCl3): δ = 25.7, 7.5, 9.1, 103.9, 30.9, 55.8, 64.2, 102.1, 111.0, 124.1, 124.2, 137.1, 135.1, 140.3, 150.0, 154.7, 155.5, 157.9, 161.3, 162.4, 162.8, 168.4 ppm; IR: ν = 1714, 1609, 1486, 1207, 1145, 1024, 835 cm⁻¹; HRMS (EI): m/z: calcd for C25H16F4NO5S: 544.1042 [M+H]+; found: 544.1049.
boxylic acid (23): Compound 22 (0.34 mmol, 0.2 g) was dissolved in THF (20 mL) and LiOH (0.04 mmol, 0.1 mL, 4.4 mmol) was added, the reaction was stirred at RT for 1 h. The solvent was evaporated and the crude product was purified by column chromatography on silica gel (first 100% EtOAc/CH2Cl2/MeOH 90:5:5 to give 23 as a yellow solid (180 mg, 91% yield). \[\text{[\text{d}]_{20} = -10 (c = 0.5 in CHCl}_3)\]. 

1H NMR (400 MHz, [D6]DMSO): \(\delta = 7.99, (d, J = 8.5 Hz, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.60 (d, J = 9.1 Hz, 1H), 7.52–7.30 (m, 4H), 6.96 (d, J = 7.1 Hz, 1H), 6.62 (d, J = 9.2 Hz, 1H), 6.48 (s, 1H), 6.44 (d, J = 2.4 Hz, 1H), 5.54–5.59 (m, 1H), 5.47 (s, 1H), 5.17–5.06 (m, 2H), 4.05–3.87 (m, 3H), 3.65–3.58 (m, 1H), 3.45–3.34 (m, 4H), 1.10 ppm (t, J = 7.2 Hz, 3H). 

13C NMR (100 MHz, [D6]DMSO): \(\delta = 169.4, 160.8, 160.1, 151.3, 153.1, 150.1, 149.5, 133.9, 127.4, 124.6, 125.8, 125.5, 125.3, 124.7, 121.1, 120.5, 113.2, 108.5, 107.3, 105.6, 105.4, 95.2, 66.5, 63.3, 44.0 (C), 31.6, 30.2 ppm (DC). 

HRMS (ESI): \(m/z = 532.2213\); \(m/z = 532.2219\); \(m/z = 532.2219\) (M+H)+; found: 532.2123.

1.5-Dioxo-8-phenyl-7-(2,1,3,5,7-pentamethylen-4,4-difluorofuro-4-bora-3a,4a-diaza-indacene-8-y)ethyl)-3,5-dihydro-2H-thiazolo[3,2-a]pyridine-3-carboxylic acid (37): Compound 37 was synthesized by following a previously published procedure,[19] starting from 36 (51 mg, 0.094 mmol). Purification by column chromatography (CH2Cl2/MeOH 97:3 to CH2Cl2/MeOH/1% AcOH 96:4:1) gave 37 as a red non-crystalline solid (27 mg, 54%). 

\[\text{[\text{d}]_{20} = -26 (c = 0.1 in CHCl}_3)\] HRNMR (400 MHz, [D6]DMSO): \(\delta = 2.23 (t, 3H), 2.35 (s, 6H), 2.44–2.68 (m, 2H), 3.05–3.29 (m, 2H), 4.85 (dd, J = 3.78 Hz, J = 8.28 Hz, 1H), 4.78 (t, J = 9.2 Hz, 1H), 4.96 (dd, J = 3.72 Hz, J = 9.21 Hz, 1H), 6.08 (s, 1H), 6.16 (s, 2H), 7.19–7.28 (m, 3H), 7.29–7.37 ppm (m, 2H); 13C NMR (100 MHz, [D6]DMSO): \(\delta = 14.1 (m, 3C), 15.6 (2C), 26.0, 33.3, 88.3, 72.8, 97.8, 107.1, 121.8, 127.3, 128.3 (2C), 130.5 (2C), 130.8 (broad), 132.8, 140.8 (broad), 145.0, 153.6 (broad), 153.9, 154.4, 158.3, 169.9 ppm; IR: \(\text{c value: c for } C}_2H_2BF_2N_3O_5; 532.2219 [M+H]+; found: 532.2213.

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<References></References>


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